Laboratory Investigations

Strontium Administration in Young Chickens Improves Bone Volume and Architecture but Does not Enhance Bone Structural and Material Strength

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Abstract. Genetic selection for rapid body growth in broiler chickens has resulted in adverse effects on the skeletal system exemplified by a higher rate of cortical fractures in leg bones. Strontium (Sr) has been reported to have beneficial effects on bone formation and strength. We supplemented the diet of 300-day-old chicks with increasing dosages of Sr (0%, 0.12%, or 0.24%) to study the capacity of the element to improve bone quality and mechanical integrity. Treatment with Sr increased cortical bone volume and reduced bone porosity as measured by micro-computed tomography. The higher level of Sr significantly reduced bone Ca content (34.7%) relative to controls (37.2%), suggesting that Sr replaced some of the Ca in bone. Material properties determined by the three-point bending test showed that bone in the Sr-treated groups withstood greater deformation prior to fracture. Load to failure and ultimate stress were similar across groups. Our results indicate that Sr treatment in rapidly growing chickens induced positive effects on bone volume but did not improve the breaking strength of long bones.

Key words: Strontium — Bone fracture — Bone quality — Broiler chicken — Mechanical property

Cortical bone fractures are one of the common skeletal disorders in fast-growing, meat-type chickens and represent an animal welfare issue as well as an economic loss during processing of the carcass [1, 2]. The rapid rate of growth in these animals, genetically selected for higher muscle mass, has been suggested to be associated with increased porosity of the cortex [2], which could explain the poorer mechanical performance of their long bones. The authors suggested that the porosity is due to rapid primary osteon formation at the periosteal surface

and the incapacity of osteoblasts to completely fill in the resultant canals, resulting in a less mechanically competent bone. It has also been reported that cortical bone from contemporary heavy strains of chicks was less well mineralized than that from slower-growing strains [3, 4]. However, when Leterrier et al. [5] reduced the growth rate by using a low-energy diet, they observed no improvement in cortical bone quality and porosity.

Recently, strontium (Sr) ranelate has been licensed in Europe for treatment of human osteoporosis. Studies in rats, mice, and monkeys have shown positive effects of the element in increasing bone volume as determined by histomophometry [6–10] and improving structural properties of bone [11]. *In vitro* studies have suggested that Sr ranelate increases bone formation by stimulating preosteoblastic differentiation and decreases bone resorption by inhibiting preosteoclasts [12–15]. The agent has also been shown to be beneficial in clinical trials by reducing the risk of bone fractures in postmenopausal osteoporotic women [16, 17]. Work by our group has also shown that Sr significantly increases both cortical and medullary bone volume in laying hens, assessed by micro-computed tomography (μCT) [18].

The present study was performed in broiler chickens to examine the effect of dietary supplementation of Sr on the structural, material, and architectural characteristics of bone. We hypothesized that Sr incorporation into bone may enhance the bone's mechanical resistance in chickens.

Materials and Methods

Animals and Treatments

Three hundred male day-old chicks were individually wing-banded and allocated equally to three dietary groups, including a standard commercial diet (control) and the same diet to which was added 0.12% or 0.24% Sr as carbonate (SrCO$_3$; Sigma-

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Aldrich, St. Louis, MO). Sr localizes in calcified tissue, and different compounds have served to provide Sr in bone metabolism studies [6, 8]. The levels of calcium (Ca) and available phosphorus (P) in the diet, according to manufacturer's specifications, were 1% and 0.45%, respectively. The Sr level of the control diet was determined to be 190 ppm. The animals were fed *ad libitum* throughout the experiment. At 3 and 6 weeks of age, 30 birds from each group were selected at random and weighed, blood samples were collected by cardiac puncture from 15 birds per treatment, animals were decapitated, and both tibiae and femurs were removed. All procedures complied with and were approved by the Pennsylvania State University Institutional Animal Care and Use Committee.

Blood Analyses

Plasma Ca was determined by flame absorption spectrophotometry (aa/ae Video 11 from Instrumentation Laboratory, Lexington, MA) [19]. Plasma inorganic P was measured colorimetrically [20] (DU 800 Spectrophotometer; Beckman Coulter, Fullerton, CA), and Sr was measured by atomic absorption spectrophotometry (AAnalyst 600; Perkin-Elmer Instruments, Foster City, CA) after dilution of plasma with 0.2% nitric acid and 0.1% Triton X-100 (Sigma-Adrich) reagent.

Bone Densitometry

Tibiae and femurs (weeks 3 and 6, $n = 30$/group) were removed, cleaned of soft tissue, and stored at $-20°C$. Bone volume was estimated by the weight change in water method [21] to calculate apparent bone density (mass per unit volume). Whole-bone mineral density (BMD) and bone mineral content (BMC) were then measured by dual-energy X-ray absorptiometry (DXA) using a Prodigy scanner (GE Lunar, Madison, WI) at the USDA-ARS Growth Biology Laboratory (Beltsville, MD). BMD measured by DXA is a projection density measurement and is expressed as grams per centimeter squared. Bones were scanned using the small animal standard mode, and individual bone results were obtained using the custom region-of-interest analysis.

Bone Microarchitectural and Morphological Analysis

A 3-mm-thick midshaft cross section was cut from the left femur using a 9" bench band saw (model 28−150; Delta Machinery, Jackson, TN). Three-dimensional (3D) data and images were collected from the diaphyseal sections using a desktop μCT system (μCT 40; Scanco Medical, Bassersdorf, Switzerland). The central 1 mm region of the sample was scanned at machine settings of 55 kVp energy, 145 μA intensity, and a 200 ms integration time. Images were reconstructed in $1,024 \times 1,024$ pixel matrices, stored in 3D arrays with an isotropic voxel size of 15.4 μm^3, and analyzed for cortical bone using a threshold value of 28% of maximum gray scale to estimate bone total volume (TV), bone volume fraction (bone volume/total volume, BV/TV), and bone surface/total volume (BS/TV). A cross-sectional image at the center of the mid-diaphyseal region of the scanned bones was also captured to further evaluate the midshaft. Using the MATLAB program (version 6.5, release 13; MathWorks, Natick, MA), the images were rotated to match the orientation used in the three-point bending tests conducted on the contralateral bones (see Bone Mechanical Tests, below). Cortical area, cross-sectional moment of inertia (CSMI) about the axis of bending, and average cortical thickness from 132 measurements around the circumference of the cortex were calculated using a program written in our laboratory.

Bone Mechanical Tests

Bone strength measurements were made at the mid-diaphysis of the right tibiae and femurs (week 6, $n = 30$/group). The bones intended for mechanical testing were wrapped in saline-soaked gauze following dissection and stored at $-20°C$. Before testing, the bones were thawed at ambient temperature and kept moist using 0.9% w/v saline solution. Three-point bending tests were conducted on bones using a MTS MiniBionix 858 testing apparatus (MTS, Eden Prairie, MN) with support spans of 60 mm (tibia) and 40 mm (femur) and a crosshead speed of 5 mm/minute under displacement control. Bones were consistently oriented for loading with the anterior cortex in tension. The proximal portion of the left femur was plotted vertically to 15 mm below the top of the femoral head in polymethylmethacrylate (Coe Tray Plastic; GC America, Alsip, IL) and secured in the MTS testing apparatus for shear testing of the femoral neck. The femoral head was loaded, parallel to the femoral shaft, at a rate of 5 mm/minute to failure. Structural properties were determined from force-displacement curves [22]. For the three-point bending test, these properties included load at yield, displacement at yield, energy absorbed at yield, ultimate load, displacement at ultimate load, energy absorbed at ultimate load, and stiffness. Stiffness was calculated as the slope of the linear portion of the load-displacement curve. The structural properties assessed for the shear test were limited to ultimate load, displacement at ultimate load, and energy absorbed at ultimate load.

To determine the material properties of the midshaft of the femur, geometrical data together with data obtained from the femoral shaft flexural tests were used to estimate the yield and failure stress ($\sigma = FLc/4I$), strain ($\epsilon = 12cd/L^2$), and modulus of elasticity ($E = FL^3/d48I$) of the bone tissue, where F is either yield or failure load, L is support span length, c is the perpendicular distance from the centroid to the periosteal tensile surface, I is CSMI, and d is the amount of bone deflection at yield or ultimate load [23]. CSMI and c were obtained from the μCT image of the contralateral femoral midshaft. Material properties of the femoral neck were not derived due to the complex loading mode at this site.

Bone Compositional Aanalyses

After mechanical testing, tibiae and femur fragments were collected and ashed in a muffle furnace at 600°C for 18 hours and then weighed to determine ash weight and bone ash percent (ash weight/dried bone weight). Ash was then dissolved in 70% nitric acid (J. T. Baker, Phillipsburg, NJ) overnight. The acid ash mixture was then measured for Ca, Sr, and P concentrations after appropriate dilutions with reagents, as described for plasma composition analyses.

Statistical Analysis

The distribution of the data was tested for normality. Data transformation or nonparametric test of Kruskal-Wallis was used for non-normal distributions when appropriate. For all normally distributed data, one-way analysis of variance (Minitab, release 14; Minitab, State College, PA) was used to investigate group differences based on Sr dietary supplement level. Results are presented as means ± standard error (SE). Significant intergroup differences for BMD, BMC, morphometric data, architectural parameters, biochemical analyses, and mechanical properties were determined with Tukey's test, and correlations were calculated using the Pearson test. Significance was defined as $P < 0.05$ in all cases.

Results

Feed Intake, Body Weight, Blood and Bone Biochemistry

Feed intake monitored on a weekly basis did not differ between dietary groups (data not shown). Body weight of the chickens was not significantly affected by dietary Sr throughout the experiment. The data for 6 weeks of age are shown in Table 1. Plasma Sr level increased in a

Table 1. Body weight and plasma and bone mineral content in chicks after 6 weeks of dietary supplementation of strontium (mean ± SE)

	Sr in diet (%)		
	0	0.12	0.24
Body weight (g) (n = 62)	2,676.6 ± 26.0	2,639.6 ± 29.6	2,615.2 ± 30.0
Plasma (n = 12)			
Total Ca (mM)	2.85 ± 0.10	3.10 ± 0.12	3.03 ± 0.07
Inorganic P (mM)	1.58 ± 0.06	1.51 ± 0.06	1.61 ± 0.09
Sr (mM)	0.018 ± 0.002[a]	0.140 ± 0.016[b]	0.300 ± 0.063[c]
Bone (femur) (n = 15)			
Ca (mg/g ash)	372.3 ± 6.2[a]	353.2 ± 2.0[a,b]	347.7 ± 4.8[b]
P (mg/g ash)	172.0 ± 10.4	163 ± 12	160.5 ± 14.4
Sr (mg/g ash)	0.62 ± 0.04[a]	42.8 ± 1.7[b]	61.6 ± 2.6[c]

[a–c] Means with different letters differ significantly ($P < 0.05$)

Table 2. Bone density, ash weight, and ash percent following Sr treatment in chickens (mean ± SE, $n = 30$/treatment)

	Sr in diet (%)		
Parameter	0	0.12	0.24
Week 3			
Femur			
Apparent density (g/cm³)	1.130 ± 0.004[a]	1.147 ± 0.004[b]	1.180 ± 0.005[c]
BMD (DXA) (g/cm²)	0.126 ± 0.001[a]	0.140 ± 0.002[b]	0.161 ± 0.002[c]
BMC (DXA) (g)	0.75 ± 0.01[a]	0.84 ± 0.01[b]	0.94 ± 0.02[c]
Ash weight (g)	0.76 ± 0.01[a]	0.77 ± 0.01[a]	0.70 ± 0.01[b]
Ash percent	46.7 ± 0.3[a]	48.7 ± 0.4[b]	46.8 ± 0.4[a]
Tibia			
Apparent density (g/cm³)	1.142 ± 0.003[a]	1.154 ± 0.004[b]	1.163 ± 0.004[b]
BMD (DXA) (g/cm²)	0.150 ± 0.001[a]	0.173 ± 0.002[b]	0.165 ± 0.003[c]
BMC (DXA) (g)	1.33 ± 0.02[a]	1.54 ± 0.02[b]	1.41 ± 0.04[a]
Ash weight (g)	1.09 ± 0.02[a]	1.06 ± 0.01[a]	0.90 ± 0.02[b]
Ash percent	46.8 ± 0.9[a]	44.1 ± 0.8[a,b]	42.3 ± 0.6[b]
Week 6			
Femur			
Apparent density (g/cm³)	1.147 ± 0.003 [a]	1.161 ± 0.002[b]	1.181 ± 0.006[c]
BMD (DXA) (g/cm²)	0.197 ± 0.002[a]	0.219 ± 0.002[b]	0.245 ± 0.003[c]
BMC (DXA) (g)	2.41 ± 0.04[a]	2.78 ± 0.06[b]	3.08 ± 0.06[c]
Ash weight (g)	2.11 ± 0.03	2.10 ± 0.04	2.10 ± 0.04
Ash percent	34.6 ± 0.3	34.2 ± 0.4	34.5 ± 0.5
Tibia			
Apparent density (g/cm³)	1.162 ± 0.002[a]	1.182 ± 0.002[b]	1.191 ± 0.005[b]
BMD (DXA) (g/cm²)	0.236 ± 0.004[a]	0.277 ± 0.003[b]	0.290 ± 0.005[b]
BMC (DXA) (g)	4.05 ± 0.07[a]	4.43 ± 0.06[b]	4.77 ± 0.06[c]
Ash weight (g)	3.45 ± 0.07	3.37 ± 0.07	3.37 ± 0.06
Ash percent	37.5 ± 0.3	38.0 ± 0.4	37.0 ± 0.4

[a–c] Means with different letters differ significantly ($P < 0.05$)

dose-dependent manner, with no effect on Ca and P levels. The higher level of Sr appeared to depress the Ca retention of bone by about 6%, while P utilization was apparently unaffected.

Densitometry and Bone Ash Measurements

DXA measurements of BMD and BMC increased significantly with Sr at both 3 and 6 weeks of age (Table 2). Significant increases were also observed in bone apparent densities measured by mass to volume ratio. At 3 weeks of age, total mineral content of bone (ash weight) in both femur and tibia was negatively affected with the higher level of dietary Sr but was restored to normal by week 6. Dry bone ash percent was reduced in tibiae at 3 weeks but returned to control levels at week 6.

Architectural and Biomechanical Measurements

As shown in Table 3, there was a significant increase in cortical bone volume fraction (BV/TV) of the femoral midshaft for both levels of Sr compared with controls.

Table 3. Skeletal properties in chickens fed different levels of Sr for 6 weeks (mean $\pm$ SE, $n = 30$/treatment)

	Sr in diet (%)		
Parameter	0	0.12	0.24
Femoral geometry and architecture			
Length (mm)	75.2 $\pm$ 0.4	75.9 $\pm$ 0.5	75.7 $\pm$ 0.5
Midshaft outer diam. (mm)	10.12 $\pm$ 0.12[a]	10.15 $\pm$ 0.14[a]	10.54 $\pm$ 0.11[b]
Midshaft inner diam. (mm)	7.1 $\pm$ 0.1	7.3 $\pm$ 0.1	7.4 $\pm$ 0.1
Cortical area (mm^2)	32.5 $\pm$ 0.8	32.3 $\pm$ 0.7	33.5 $\pm$ 0.9
Medullary area (mm^2)	42.60 $\pm$ 1.08	44.1 $\pm$ 1.2	45.30 $\pm$ 1.05
Total area (mm^2)	75.2 $\pm$ 1.5	76.5 $\pm$ 1.7	79.4 $\pm$ 1.4
Average thickness (mm)	1.06 $\pm$ 0.02	1.04 $\pm$ 0.02	1.05 $\pm$ 0.03
CSMI (mm^4)	331.6 $\pm$ 14.6	335.6 $\pm$ 15.4	365.5 $\pm$ 14.6
Tensile distance (mm)	4.72 $\pm$ 0.06[a]	4.71 $\pm$ 0.07[a]	4.98 $\pm$ 0.07[b]
μCT TV	24.2 $\pm$ 0.9	25.7 $\pm$ 0.8	25.7 $\pm$ 1.0
μCT BV/TV	0.877 $\pm$ 0.007[a]	0.917 $\pm$ 0.005[b]	0.931 $\pm$ 0.003[b]
μCT BS/BV	5.0 $\pm$ 0.6[a]	3.1 $\pm$ 0.3[b]	3.0 $\pm$ 0.4[b]
Bending test of femur			
Load at yield (n)	228.9 $\pm$ 9.1	219.2 $\pm$ 10.8	222.1 $\pm$ 12.0
Displacement at yield (mm)	1.12 $\pm$ 0.05	1.16 $\pm$ 0.05	1.26 $\pm$ 0.06
Work to yield (N.mm)	141.8 $\pm$ 9.8	143.2 $\pm$ 12.0	156.5 $\pm$ 14.4
Ultimate load (N)	310.3 $\pm$ 9.3	297.3 $\pm$ 8.4	304.0 $\pm$ 11.2
Displacement at ultimate load (mm)	2.25 $\pm$ 0.08[a]	2.25 $\pm$ 0.09[a]	2.58 $\pm$ 0.12[b]
Work to ultimate load (N.mm)	444.9 $\pm$ 22.8	432.9 $\pm$ 23.1	466.5 $\pm$ 24.0
Stiffness (N/mm)	231.6 $\pm$ 9.2[a]	213.5 $\pm$ 8.5[a,b]	199.2 $\pm$ 8.8[b]
Yield stress (Mpa)	33.90 $\pm$ 1.68	32.3 $\pm$ 1.9	30.2 $\pm$ 1.2
Ultimate stress (Mpa)	44.1 $\pm$ 1.7	43.4 $\pm$ 1.7	40.9 $\pm$ 1.4
Yield strain	0.040 $\pm$ 0.002[a]	0.041 $\pm$ 0.002[a]	0.048 $\pm$ 0.003[b]
Ultimate strain	0.082 $\pm$ 0.004[a]	0.080 $\pm$ 0.004[a]	0.093 $\pm$ 0.004[b]
Elastic modulus (GPa)	0.837 $\pm$ 0.042[a]	0.783 $\pm$ 0.043[a]	0.674 $\pm$ 0.035[b]
Femoral neck shear test			
Ultimate load (N)	132.9 $\pm$ 4.6	126.4 $\pm$ 5.9	119.4 $\pm$ 4.4
Displacement at ultimate load (mm)	2.53 $\pm$ 0.12	2.52 $\pm$ 0.20	2.95 $\pm$ 0.20
Work to ultimate load (N.mm)	190.6 $\pm$ 11.9	196.9 $\pm$ 17.1	211.4 $\pm$ 16.2
Structural properties of tibia			
Load at yield (N)	252.3 $\pm$ 16	252.6 $\pm$ 14.1	220.5 $\pm$ 15.7
Displacement at yield (mm)	1.16 $\pm$ 0.07	1.19 $\pm$ 0.07	1.09 $\pm$ 0.06
Work to yield (N.mm)	159.9 $\pm$ 19.1	176.3 $\pm$ 20.9	141.7 $\pm$ 20.4
Ultimate load (N)	376.3 $\pm$ 15	376.8 $\pm$ 10.8	389.6 $\pm$ 12.2
Displacement at ultimate load (mm)	2.44 $\pm$ 0.06[a]	2.40 $\pm$ 0.06[a]	2.65 $\pm$ 0.05[b]
Work to ultimate load (N.mm)	567.0 $\pm$ 26.1	549.7 $\pm$ 27.1	615.9 $\pm$ 20.6
Stiffness (N/mm)	238.0 $\pm$ 8.2[a]	237.5 $\pm$ 7.1[a]	209.5 $\pm$ 6.4[b]

[a–c] Means with different letters differ significantly ($P < 0.05$)
N.mm = N times mm (N $\times$ mm); N/mm = N divided by mm

Testing mechanical competence of bone showed that there were decreases in both femur and tibia stiffness. There was also a decrease in elastic modulus and an increase in yield and ultimate strain of femur with 0.24% dietary Sr. Both the femur and tibia showed considerably greater deformation in bending as a result of Sr. Although not statistically significant, similar increases in femoral neck shear deformation and corresponding energy absorption were observed with the higher level of dietary Sr. No other notable trends in structural or material parameters as a function of Sr dosage were found.

Discussion

The dose-dependent increase of Sr levels in plasma and bone (Table 1) was indicative of the Sr content of the diet and a high degree of absorption and retention of the dietary Sr. The experimental levels of dietary Sr were determined by a series of pilot studies to establish levels which would increase bone density without any rachitogenic effect on bone [24] or adverse effect on body weight of chicks. These levels are considered low [25] and comparable with those used in studies with rats [11].

Values of BMD and BMC as measured by DXA and bone density as determined by the mass per volume method increased significantly as a result of Sr supplementation in the diet of chicks (Table 2). Previous reports indicate that the presence of Sr in bone, because of its higher atomic number compared with Ca (38 vs. 20), causes stronger X-ray attenuation and therefore overestimates both BMD and BMC measured by DXA densitometers [26, 27]. When using an 8% correction

factor for overestimated BMD, as suggested for each 1% molar fraction of Sr in bone [27], values of BMD and BMC dropped to levels that were not significantly different from those of the control group. However, the increase in apparent density of the bones as measured by the conventional method of mass per unit volume (Table 2) indicates that Sr effectively enhanced density in both 3- and 6-week-old chickens. It might be possible that the factor needed to properly adjust our DXA indices for Sr content is lower than the value used.

The results indicate that a dose of 0.24% dietary Sr depressed bone mineralization during the earlier, more rapid phase of bone growth (3 weeks of age). The reduced ash weight of bones and a decrease in tibial ash percentage at this age support this perspective. The ash percentage of femoral bone increased with 0.12% dietary Sr but then dropped back to normal with 0.24% dietary Sr. These data are in agreement with previous reports in rats [6, 28–30] indicating that high doses of Sr induced changes in bone mineralization documented by decreased bone growth, bone ash measurements, and Ca content. The differences in response of the tibia and femur to Sr (Table 2) could be due to a different pattern of bone development and mineralization rate between the femur and tibia [31]. This is also observed in DXA measurements where the femur appears to be more responsive than the tibia to increasing doses of Sr.

Ash measurements were restored to normal at 6 weeks of age (Table 2). This was likely due to a reduced Sr intake. When adjusted for body weight and feed consumption, dietary Sr intake of 0.24% was equivalent to 3.5, 2.1, and 1.8 mmol Sr/kg body weight daily during the first, third, and sixth weeks of age, respectively. In addition, the rate of bone growth is slower at week 6 compared to week 3 and there is also an increased discrimination of Sr in favor of Ca at the intestinal level with age [32]. Nevertheless, the substitution of Sr for Ca in bone at the higher level of dietary Sr was evident in 6-week-old chickens. Bone Ca content was reduced from 37.2% in control birds to 34.7% in birds receiving 0.24% Sr in the diet (Table 1). Lack of difference in apparent femur density between 3- and 6-week-old chicks on 0.24% dietary Sr also suggests that at least some of the benefit of Sr in enhancing the density of bone was lost by concomitant loss of Ca. Colvin et al. [33] also reported a significant reduction in bone ash at the expense of bone Ca when they gradually replaced Ca with Sr in the diet of growing chicks. They attributed the reduced bone ash content to the lack of mineralization at the growth plates of the bone. The decrease in bone Ca with high Sr intake may result from reduced Ca uptake at both the intestinal and bone levels. A high level of Sr, e.g., has been reported to inhibit vitamin D_3-induced Ca-binding protein and Ca absorption in intestine [34]. Although comparable to other animal studies [11, 25], the doses used in this experiment may seem to be high given the rapid bone calcification in chickens and the short duration of the study.

We analyzed BV/TV as measured by μCT to study the ability of Sr to reduce the porosity of cortical bone by enhancing bone formation, as has been suggested by Marie et al. [25]. A significant increase in cortical bone volume fraction with Sr treatment was present. The increment was most likely due to decreased porosity, evidenced by a significant increase in bone volume, whereas total volume was unchanged. A significant reduction in BS/BV is also indicative of a decrease in porosity with both levels of Sr used. The increase in BV as measured by μCT is in agreement with DXA results and in disagreement with ash weight and ash percent. It is likely that any increment in bone mass was too small to be detected by bone ash measurement. It is also possible that BV measurements might be overestimated by μCT, as is bone density in DXA; however, the magnitude of the overestimation by μCT is expected to be minimal compared with DXA. The μCT results presented in Table 3 are not based on a calibrated phantom, as is the case of BMD and BMC by DXA, but rather an assigned density threshold. It is recognized that there could be an edge effect on the μCT measurements, particularly the BS measurements; but it is unlikely that this effect is responsible for the significant differences identified for BV/TV or differences in femoral cross-sectional geometry. The outer diameter of the femoral cross section in the anterior-posterior plane was increased with 0.24% Sr ($P = 0.04$) with a corresponding nonsignificant trend in CSMI. This might be indicative of cortical expansion with Sr, although the tendency to increase of total volume and total area was not significant ($P = 0.14$). Further examination of μCT data is limited due to the lack of histomorphometric analyses.

Although no significant differences were identified for femur structural strength as a function of Sr dietary level, a significant correlation between maximum load and BV/TV was observed ($r = 0.62$, $P < 0.001$). We did not observe any change in yield and ultimate bending load as a result of Sr. It is possible that the variability inherent in the mechanical analysis may have precluded detection. A significant increase in the deformation of the femur and tibia at ultimate load, reduced stiffness, and increased absorbed energy (although not statistically significant) with the higher level of Sr indicates that the bone formed under Sr treatment is more pliable. Further evidence of this was seen with a reduction in elastic modulus and an increase in both yield and ultimate strain of femoral bone as a function of Sr level. Such an effect might be attributed to increased osteoid formation [24, 35]. Other than increase in organic percentage, one possible explanation of this could be physiochemical interference of Sr, at high levels, with the hydroxyapatite formation and crystal properties [36].

In conclusion, we show that treatment of rapidly growing chickens with Sr appears to produce large and significant increases in BMD and BMC as measured by DXA but that these changes could be due to enhanced X-ray attenuation by Sr and, to a lesser extent, to enhanced bone deposition. However, apparent bone density increased significantly with Sr, providing support for the positive effects of the element. An increase in BV/TV and a decrease in BS/BV of the femoral midshaft measured by μCT also suggest a positive influence of Sr on cortical bone formation and a reduction in bone porosity. No significant differences were identified for yield or ultimate load, but there was a significant decrease in stiffness and an increase in strain of bone at the higher level of Sr treatment. The positive influence of Sr on bone strength in humans [16, 37] is primarily on trabecular bone, an effect that may be less pronounced in cortical bone and could explain some of our observations on mechanical parameters.

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